IL 10-2002-019
In Reply Refer To: 13B

December 13, 2002

UNDER SECRETARY FOR HEALTH'S INFORMATION LETTER

DIAGNOSTIC TESTING FOR HEPATITIS C VIRUS

1. Purpose. This Under Secretary for Health's Information Letter provides suggested algorithms for the diagnosis of hepatitis C virus (HCV) infection and notification of related test results.

2. Background

a. The Department of Veterans Affairs (VA) is currently engaged in the largest program of screening and testing for HCV infection in the country. In the past three years, over 1.7 million patients who use VA medical services have been screened for hepatitis C risk factors. In fiscal year 2001, over 480,000 hepatitis C antibody tests were performed.

b. Appropriate counseling and assessment, leading to a decision about a plan of care, must follow a positive diagnostic test for hepatitis C.

c. An appropriate plan of care may be defined as watchful waiting, drug therapy, and/or treatment for co-morbidities or inter-current illnesses.

d. Clinicians must receive accurate and complete results that allow them to determine if patients: (1) have truly been infected with hepatitis C, and (2) have chronic (active) infection.

3. Definitions

a. **Diagnostic Test for HCV.** One or more laboratory tests that confirm that a patient has been exposed to hepatitis C in the past or currently has active infection with hepatitis C. Such laboratory tests are:

(1) Serologic tests that confirm presence, in the patient's blood, of antibody to HCV (anti-HCV); and

(2) Tests that assess presence of HCV in the patient's blood.

b. **Enzyme immunoassays (EIA).** EIA detects the presence of anti-HCV in the serum. These tests are commonly used for initial screening for hepatitis C. The EIA does not differentiate between acute, chronic, or resolved infection. The EIA is highly sensitive, but may yield false negative results in the window period between exposure and seroconversion (the window period varies, but averages 8 weeks), or in patients with immunologic disorders that impair production of antibody. The EIA may be falsely positive due to cross-reactivity with other antigens. On a population basis, the false positive rate of the EIA increases as the prevalence of hepatitis C antibody positivity decreases. EIA results are expressed as "positive" or "negative," usually accompanied by an optical density (O.D.) reading.

c. **Recombinant Immunoblot Assay (RIBA).** RIBA is a very specific test performed using antigens from the HCV genome combined with the patient's serum. The RIBA can be used to confirm a positive EIA in certain clinical scenarios, such as the patient with a positive EIA and negative test for HCV in the blood. RIBA results are reported as positive or negative. Indeterminant results are also reported, when partial reactivity occurs between the test antigens and proteins in the patient's serum. Indeterminant results are best resolved by performing an HCV RNA test.

d. **HCV Ribonucleic Acid (RNA).** HCV RNA tests detect the presence of HCV in the patient's blood, indicating an active infection.

(1) Qualitative HCV RNA Tests. Qualitative HCV RNA tests detect the presence or absence of HCV and are reported as "positive" or "negative."

(2) Quantitative HCV RNA Tests. Quantitative HCV RNA tests measure the amount of virus in blood plasma. Results are expressed as viral RNA copies per milliliter (ml) of plasma or as International Units per ml (IU/ml) of plasma.

e. **EIA signal to Cutoff Ratio.** The EIA signal to cutoff ratio is a comparison of the optical density of the patient's positive EIA result to the optical density of the laboratory's positive EIA control. If the ratio is ≥ 3.8 (using the most widely employed diagnostic kits), the predictive value (that the patient truly has HCV antibody in the blood) of the patient's positive result is high. The predictive value of signal to cutoff ratios using newer assays such as the Vitros Eci has not been established. The 3.8 value should only be used with the Ortho 3.0 or Abbott 2.0 assays.

f. **HCV Genotype Tests.** HCV Genotype tests determine which of six known genetically distinct types of HCV are found in the patient's blood. Knowledge of HCV genotype is important when considering antiviral therapy, as patients with genotypes two and three are twice as likely to achieve a sustained viral response as those with genotype one. In addition, duration of treatment may vary with genotype. Patients with genotypes two and three are usually treated for 24 weeks with combination alpha interferon and ribavirin; those with genotype one usually require 48 weeks of therapy.

g. **Biochemical Indicators of HCV**

(1) Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) indicate liver damage and may range from 0 to 20 times the upper limit of normal; ALT is usually greater than AST, but this finding may be reversed in cirrhosis.

(2) Alkaline phosphatase is usually normal; elevation may indicate cirrhosis.

(3) Gamma glutamyl transpeptidase (GGTP) is usually normal; elevation may indicate cirrhosis.

(4) Rheumatoid factor may be present in cirrhosis.

(5) Low platelet count and white blood cell count may be present in cirrhosis.

(6) Albumin level and prothrombin time are normal until late-stage.

(7) Iron and ferritin may be slightly elevated.

4. Guidance

a. The Hepatitis C Technical Advisory Group and the VA Hepatitis C Resource Centers have formulated suggested algorithms for diagnostic testing and for notification of test results. Several alternatives are presented for both testing and notification. Clinicians, in consultation with Pathology and Laboratory Medicine (115), liver specialists, and other interested parties at the local and Veterans Integrated Service Network level may work together to choose the best algorithms for their site.

b. Algorithms that address the issue of confirmatory testing take advantage of the EIA signal to cut-off ratio as a means of identifying "low positive" results that are likely to be false positives because of cross-reactivity with other antigens.

c. Any of the following three choices is a reasonable approach. The choice of any one of them might depend on local resources and preferences of clinicians and laboratory administrators. ***NOTE:*** *Diagnostic Testing Algorithms are depicted in Attachment A.*

(1) **Diagnostic Testing Algorithm #1.** When a diagnostic test for hepatitis C is ordered, specimens for EIA and RIBA will be collected and, initially, only the EIA will be performed. For those results where the EIA signal to cut-off ratio (using the Ortho 3.0 or Abbott 2.0 assay) is positive, but < 3.8, a RIBA will automatically be performed without an additional order. The diagnostic test for hepatitis C will be reported as positive if the EIA signal to cut-off ratio is ≥ 3.8 or if the RIBA is positive. HCV-RNA tests will be performed only with an additional specific order from a clinician and will require collection of an additional specimen. This algorithm requires modification if EIA assays other than those mentioned above are used.

(2) **Diagnostic Testing Algorithm #2.** When a diagnostic test for hepatitis C is ordered, specimens for EIA and RIBA, as well as HCV RNA will be collected. ***NOTE:*** *Most laboratories would choose the qualitative HCV RNA in this circumstance. The choice between qualitative and quantitative HCV RNA tests might be governed by relative costs of different assays available, as well as differences in their limits of detection.* If the EIA signal to cut-off ratio (using the Ortho 3.0 or Abbott 2.0 assay) is ≥ 3.8, the HCV RNA test will be performed. If the EIA signal to cut-off ratio is positive, but < 3.8, a RIBA will be performed. If the RIBA is positive, the HCV RNA will be done. The report of a positive test will include the EIA result and the RIBA result (if done), as well as the HCV RNA result. This algorithm requires modification if EIA assays other than those mentioned above are used.

(3) **Diagnostic Testing Algorithm #3.** When a diagnostic test for hepatitis C is ordered, specimens for EIA, RIBA, and HCV RNA will be collected. ***NOTE:*** *Most laboratories would choose the qualitative HCV RNA in this circumstance. The choice between qualitative and quantitative HCV RNA tests might be governed by relative costs of different assays available, as*

well as differences in their limits of detection. If the EIA is positive, regardless of the optical density reading, the HCV RNA will be done. If the HCV RNA is undetectable, a confirmatory RIBA is done. If the HCV RNA is detectable, no confirmatory RIBA is performed.

d. Systems need to be in place to call a clinician's attention to test results, because hepatitis C testing is frequently performed in the outpatient setting on patients who appear clinically well. Several systems are available; the choice of the best system depends on local resources and workload. Some possible systems for notifying clinicians of possible test results:

(1) **Notification System #1.** A positive diagnostic test for hepatitis C is treated as a "panic" value. The laboratory generates a phone call to the ordering clinician. This system has the advantage of ensuring notification and calling attention to the result. It may, however, create substantial work for the laboratory and in facilities with large numbers of trainees or part-time clinical staff it may be difficult to locate the ordering clinician.

(2) **Notification System #2.** A positive diagnostic test for hepatitis C triggers a "view alert" in the Veterans Health Information Systems and Technology Architecture (VistA) Computerized Patient Record System (CPRS) for the ordering clinician or responsible physician. This system is commonly used for abnormal radiology results and laboratory abnormal/critical values. Some programming is required to utilize the View Alert system (which is designed for numeric results) with hepatitis C serology, which is usually reported in non-numeric (positive/negative) manner. Configuration of the laboratory test entry in file 60 can be performed locally by the facility's Laboratory Implementation Manager. Briefly, this will involve utilizing either a delta check or recording positive and negative results as numeric equivalents (0 and 1, e.g.). Assistance in making the correct changes may be obtained from the National Vista Support Help Desk, 888-596-4357. The advantage of this process is that it can be largely automated. The disadvantage is that clinicians who receive many clinical alerts may not give alerts adequate attention. If a clinical alert is viewed and dismissed, the clinician may not remember it at the appropriate time.

(3) **Notification System #3.** All diagnostic test results for hepatitis C are reported in VistA CPRS and to a single designated individual, such as a hepatitis C coordinator, primary care case manager, or another locally designated individual. The designated individual has responsibility for ensuring that patients with positive tests are notified and that proper clinical assessments take place. While this is a system that maximizes accountability and follow up, it may consume significant personnel time in a facility with a large number of tests.

e. Facility Directors are encouraged to provide copies of this Information Letter to laboratory leadership and staff, hepatitis C lead clinicians, hepatitis C coordinators, infectious diseases clinicians, Infection Control practitioners, Primary Care Teams, ambulatory clinic staff, community-based outpatient clinics.

5. References

a. VHA Directive 2001-009, National Hepatitis C Program, February 27, 2001.

b. VHA Directive 2002-022, Implementation of National Hepatitis C Case Registry, April 11, 2002.

c. National VA Hepatitis C website: www.va.gov/hepatitisc

d. National Institutes of Health (NIH)-National Institute of Diabetes and Digestive and Kidney Diseases(NIDDK) Chronic Hepatitis C : Current Disease Management: www.niddk.nih.gov/health/digest/pubs/chrnhepc/chrnhepc.htm

e. Courouce AM, Janot C. "Recombinant Immunoblot Assay First and Second Generations on 732 Blood Donors Reactive for Antibodies to Hepatitis C Virus by ELISA. The Hepatitis Study Group of the French Society of Blood Transfusion," Vox Sanguinis 1991; 61:177-80.

f. Sakugawa H, Nakasone H, Nakayoshi T, Kinjo F, Saito A, Yakabi S, Sukeren H, et al. "High Proportion of False positive Reactions Among Donors with Anti-HCV Antibodies in a Low Prevalence Area" Journal of Medical Virology (J Med Virol) 1995; 46: 334-8.

g. dos Santos VA, Azevedo RS, Camargo ME, Alves VA: "Serodiagnosis of Hepatitis C Virus. Effect of New Evaluation of Cutoff Values for Enzyme-linked Immunosorbent Assay in Brazilian Patients," American Journal of Clinical Pathology (Am J Clin Pathol) 1999; 112:418-24.

6. Contact. Questions regarding this Information Letter may be addressed to the Director, National Hepatitis C Program (13B) at (203) 932-5711 ext. 3738 or (202) 273-8567.

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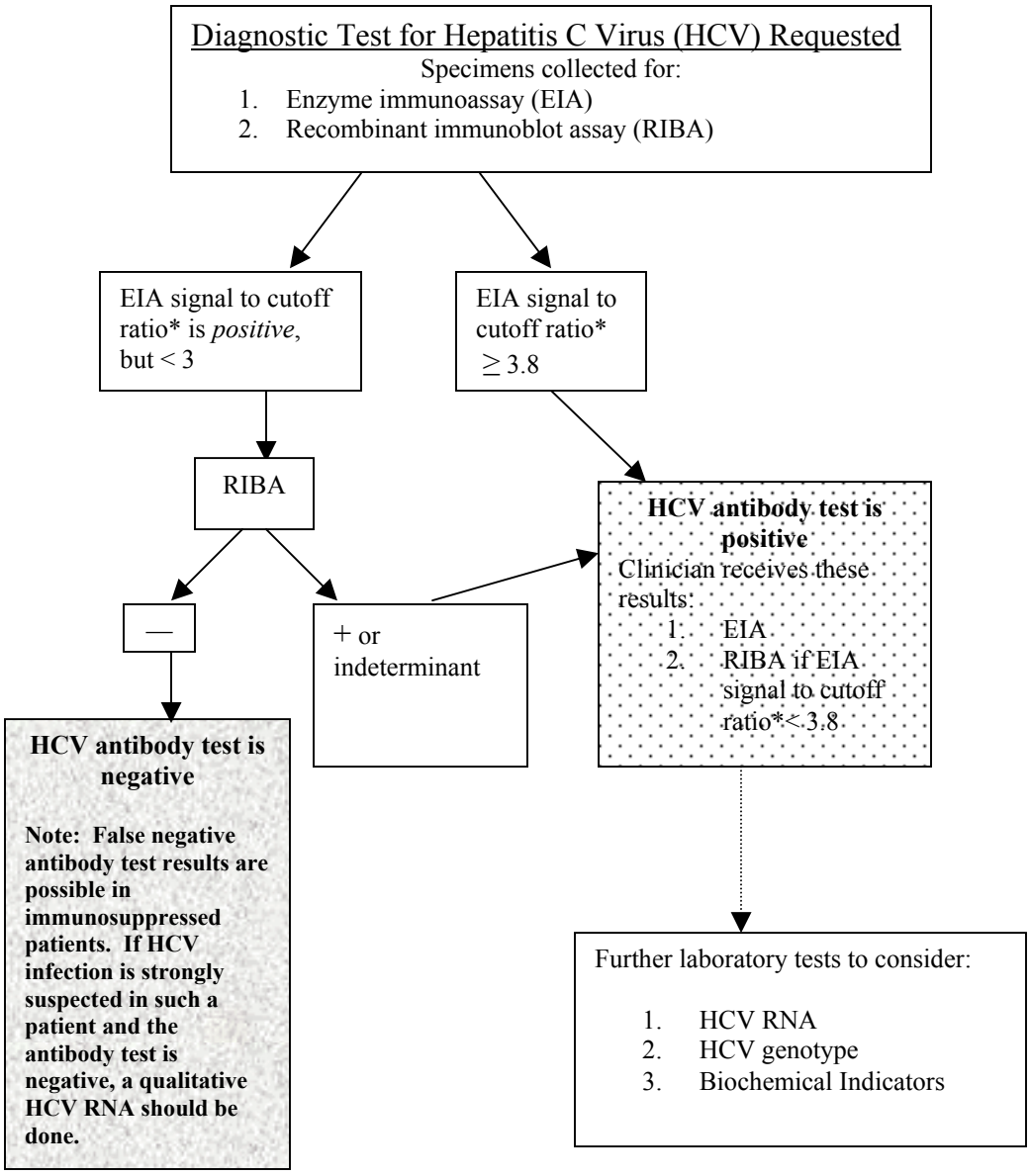
ATTACHMENT A

DIAGNOSTIC TESTING ALGORITHM #1



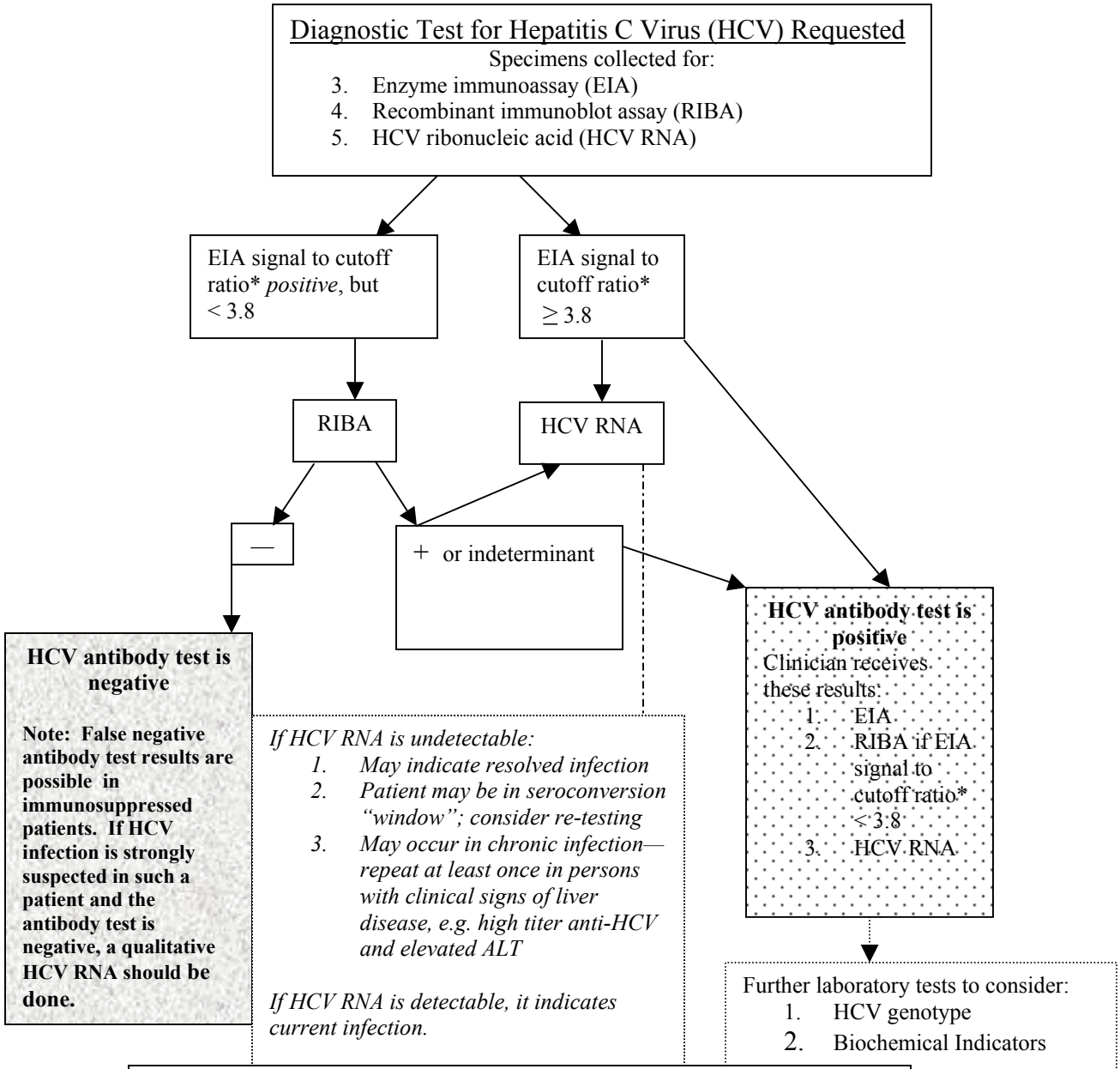
Summary of Algorithm #1 Outcomes:

1. EIA signal to cutoff ratio* *positive*, but < 3.8, RIBA negative→HCV antibody negative
2. EIA signal to cutoff ratio* *positive ,* but < 3.8, RIBA positive→HCV antibody positive
3. EIA signal to cutoff ratio* ≥ 3.8→HCV antibody positive

Note: Ascertainment of resolved vs. chronic infection requires HCV RNA.
Note: Development of a plan of care for chronic infection requires biochemical indicators, HCV genotype, and other clinical assessments.

**The 3.8 value should only be used with Ortho 3.0 or Abbott 3.0 assays.*

DIAGNOSTIC TESTING ALGORITHM #2

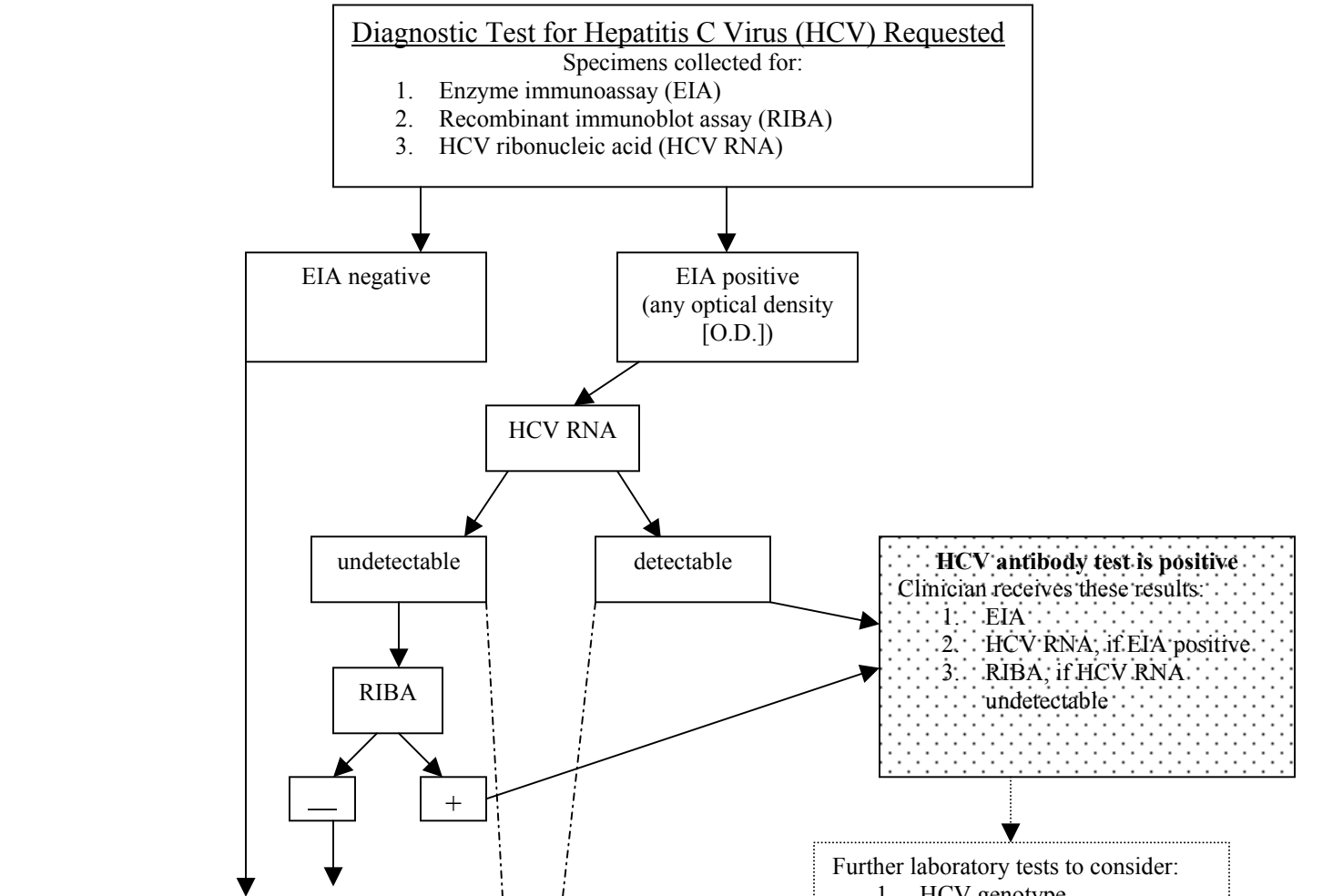


Summary of Algorithm #2 Outcomes:

1. EIA signal to cutoff ratio* *positive*, but < 3.8, RIBA negative→HCV antibody negative
2. EIA signal to cutoff ratio* *positive*, but < 3.8, RIBA positive, HCV RNA negative→resolved HCV infection (likely)
3. EIA signal to cutoff ratio* *positive*, but < 3.8, RIBA pos, HCV RNA positive→chronic HCV infection
4. EIA signal to cutoff ratio* ≥ 3.8, HCV RNA negative→resolved HCV infection or seroconversion "window", or chronic HCV with fluctuating viral load
5. EIA signal to cutoff ratio* ≥ 3.8, HCV RNA positive→chronic HCV infection

Note: Ascertainment of resolved vs. chronic infection requires HCV RNA.
Note: Development of a plan of care for chronic infection requires biochemical indicators, HCV genotype, and other clinical assessments.
A-2 **The 3.8 value should only be used with Ortho 3.0 or Abbott 3.0 assays.*

DIAGNOSTIC TESTING ALGORITHM #3



HCV antibody test is negative

Note: False negative antibody test results are possible in immunosuppressed patients. If HCV infection is strongly suspected in such a patient and the antibody test is negative, a qualitative HCV RNA should be done.

If HCV RNA is undetectable:

1. *May indicate resolved infection*
2. *Patient may be in seroconversion "window"; consider re-testing*
3. *May occur in chronic infection—repeat at least once in persons with clinical signs of liver disease, e.g. high titer anti-HCV and elevated ALT*

If HCV RNA is detectable, it indicates current infection.

Summary of Algorithm #3 Outcomes:

1. EIA negative→HCV antibody negative
2. EIA positive at any O.D, HCV RNA undetectable, RIBA negative→false positive EIA
3. EIA positive at any O.D, HCV RNA undetectable, RIBA positive→ resolved HCV infection or seroconversion "window", or chronic HCV w/fluctuating viral load
4. EIA positive at any O.D, HCV RNA detectable→chronic HCV infection

Note: Ascertainment of resolved vs. chronic infection requires HCV RNA.
Note: Development of a plan of care for chronic infection requires biochemical indicators, HCV genotype, and other clinical assessments.